EVALUATION OF THE PHYSIOLOGICAL RESPONSES OF QUINNAT AND SOCKEYE SALMON TO ACUTE STRESSORS AND SAMPLING PROCEDURES.

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ABSTRACT

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Salmon (*Oncorhynchus tshawytscha* and *O. nerka*) were subjected to various stressors: agitation, high temperature, anaesthetization and aerial exposure, serial sampling of fish, and transportation. Most stressors produced a marked increase in circulating levels of cortisol which fell back to control values relatively quickly once the stressor was removed. Anaesthetization and aerial exposure, and serial sampling did not produce changes in plasma cortisol. In freshwater, an increase in cortisol levels was accompanied by a fall in sodium, chloride and plasma osmolarity plus an increase in haematocrit. In seawater, an increase in plasma cortisol was associated with increases in plasma sodium, chloride, osmolarity and haematocrit.

KEYWORDS: stress - salmon - cortisol.

INTRODUCTION

Changes in a variety of haematological parameters such as plasma cortisol, glucose and haematocrit have been used to reliably characterise and quantify stress responses in fish (Carmichael *et al.* 1984, Schwalme & Mackay 1985, Wedemeyer & Yasutake 1987, White & Fletcher 1986). Obtaining blood from fish results in an additional, acute stress response caused by the sampling procedures (i.e., net capture, anaesthesia, and blood extraction), so it is essential that the time course of changes in the various investigated parameters induced by handling is known. Once the time course is known, sampling stressors can be distinguished from the actual stressors examined. A number of studies have examined the stress response of fish to anaesthetics, sampling and handling (Barton *et al.* 1980, Oikari & Soivio 1975, Strange & Schreck 1978, Strange *et al.* 1977). However, as sampling techniques, maintenance, and the type, or stock of fish used can vary greatly, it is important to validate the methods used in any given experiment in order to be confident that the sampling methods do not significantly affect the results. Knowledge of both the immediate and long term responses of a fish to a stressor may be needed. When controlling for blood sampling procedures, measured variables that show an immediate response to the stressor (i.e., those that have the fastest rise time) are the best indicators. Conversely, the best indicators of recovery from a stressor are variables that take the longest time to return to pre-stress levels.

The objectives of the present study were to characterise the stress responses to handling of quinnat salmon (*Oncorhynchus tshawytscha*) in fresh water and sea water, and to validate experimental handling procedures (blood sampling and transportation) for both quinnat and sockeye salmon (*Oncorhynchus nerka*). Changes in plasma cortisol were used to indicate the primary stress response (Mazeaud *et al.* 1977) and a variety of secondary stress effects including ionic and osmotic changes were monitored.

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MATERIALS AND METHODS

FISH STOCKS

Sockeye (*Oncorhynchus nerka*) and quinnat (*Oncorhynchus tshawytscha*) salmon (0+ year) were obtained from the M.A.F. Glenariffe Research Hatchery and transported to either the Zoology Department, University of Canterbury, Christchurch, or to the Edward Percival Field Station, Kaikoura, where they were kept in large 80 l, opaque aquaria at a density no greater than 6-10 g fish per litre. The animals were maintained in a photoperiod of 12 hr light : 12 hr dark at the University of Canterbury, and in a natural photoperiod at the Field Station. Fish were kept in freshwater (termed freshwater acclimated), or they were transferred into seawater. All fish survived the transfer and were termed seawater acclimated. Details of physiological changes occuring during transfer into seawater can be found in Franklin *et al.* 1990a, b). Fish were maintained for at least 7 days before being used and were not fed during the experimental trials. All experiments were started between 0800 and 0900 h.

SAMPLING PROCEDURES

To sample, salmon were rapidly netted out of the aquaria and placed into the anaesthetic, 2-phenoxyethanol, which was diluted with 30% seawater to a concentration that immobilised the fish within 30 seconds (4-6 ml l^{-1}). The dilute seawater was used to approximate the ionic conditions present in the plasma. Thus any changes to gill membrane permeability brought about by the anaesthetic would have little effect on ion exchange. The fish were taken from the anaesthetic, and excess water removed by blotting with paper towels. The caudal fin was severed with a sharp scalpel and blood was collected from the caudal vessels into a 1 ml heparinised (ammonium heparinate) hypodermic syringe. The extracted blood was transferred into a 400 μl Eppendorf centrifuge vial and a sub-sample immediately withdrawn into a micropipette for haematocrit determination by centrifugation at 20 000 g for 3 minutes. The remainder of the sample was centrifuged for 3 minutes at 5000 g. Plasma was then withdrawn and stored in vials at -80°C until analyzed. The plasma samples were analyzed for cortisol, osmolarity, sodium and chloride. Plasma cortisol was measured by radioimmunoassay (RIA) using tritiated cortisol ([1,2,6,7-3H] cortisol, Amersham International) and antiserum from Bioanalysis Ltd (U.K.). The RIA technique employed was a modification of a protocol supplied by Bioanalysis Ltd. Details of the assay can be found in Franklin (1989) or can be obtained from the authors. Cortisol (radioactive and non-radioactive) was incubated with antiserum for 2 h at room temperature. Free cortisol was then removed using dextran coated activated charcoal, leaving the antiserum bound cortisol in solution. Optimum concentration of charcoal for salmon plasma was determined to be 1 mg per assay tube. Radioactivity of the supernatant was determined by liquid scintillation. Assay sensitivity was 2.5 ng cortisol per ml of plasma using 10 μl samples. Cross reactivities with 11-deoxycortisol and cortisone were 3.3% and 0.4% respectively (as provided by Bioanalysis). These are regarded as the major steroids, other than cortisol, present in salmonid plasma (Idler & Truscott 1972, Sandor 1979). The mean intra-assay and interassay coefficients of variation for the RIA were 6.4% (n = 4) and 10.2% (n = 6), respectively. Chloride concentrations were determined from duplicate 5-10 μl plasma samples with a CMT 10 Radiometer chloride titrator. Sodium concentrations were obtained from duplicate 5 μl plasma samples diluted with 5 ml water and analyzed by emission spectrophotometry (Varian Techtron 1200 Atomic Absorption spectrophotometer). Osmolarities were measured with a vapour pressure osmometer (Wescor 5100C osmometer) using duplicate 8 μl plasma samples.

Unless noted otherwise, these sampling methods were used for the experiments outlined below. No more than 8 fish were sampled at any one time and sampling was completed within 10 min from the time of net capture.

CONFINEMENT AND PHYSICAL DISTURBANCE

Quinnat salmon (mean weight = 26.7 ± 8.5 g) were transferred from the 80 l aquaria into small plastic aquaria that had barely enough water to cover the dorsal fin of the salmon. The fish remained in these aquaria for 10 min during which time they were continually agitated by a

plastic net that was moved back and forth across the aquarium. The salmon were then transferred back into the original aquaria. Blood samples were taken before, and 1, 2, 4, 12, 24, and 48 hr after the confinement and physical disturbance. This experiment was carried out using both freshwater- and seawater-acclimated salmon. No mortality occurred during the stressor period or recovery.

SEVERE HEAT STRESS

The effect of a sudden heat shock as an acute stressor to sockeye salmon was investigated. Freshwater sockeye (mean weight = 18.3 ± 4.4 g) were netted and transferred from an aquarium set at 16°C to one set at 35°C where they remained for 10 min before being placed back into the original temperature to recover. Samples were taken before, and 1, 3, 12, 24, and 48 hr after the heat treatment. Some mortality occurred during the 48 hr recovery period. Of the 37 fish stressed, 5 died within the first hour, and 3 died between 24 and 48 hr after treatment.

SAMPLING FISH

In any experimental situation there is a problem of how to serially sample fish which are resident in a single aquarium tank. Two methods can be used: (a) all of the fish can be netted at the same time and placed in anaesthetic; or (b) fish can be netted as required. The first method has potential problems as there is a time gap between sampling the first fish out of the anaesthetic and sampling the last. The latter method has the potential problem of stressing fish due to disturbance while netting the initial animals. The following experiments were designed to test these procedures.

For both species, 7 salmon were netted and then anaesthetized. The salmon were removed from the anaesthetic, placed onto a moist paper towel, and then serially sampled at 1, 3, 5, 8, 12, 15, and 25 min after the initial net capture. This procedure was repeated 3 times for each species.

To evaluate the effect of the repeated sampling of fish held in the same aquarium, subgroups of salmon were sampled at 0, 1, 2, 12, and 24 hrs. Thus fish in later sub-groups were disturbed as earlier sub-groups were sampled.

TRANSPORTATION

The effect of transportation was examined in both sockeye and quinnat salmon. Fish were transported from the Zoology Department, University of Canterbury, to the Edward Percival Field Station, Kaikoura, in March 1985. Fish were netted from the Zoology Department aquaria and placed into large plastic bags filled with oxygen saturated water. The fish were stocked at a density of 20-30 g fish per litre. The bags were sealed with an oxygen filled space and transported by road to Kaikoura. The water temperature in the plastic bags was initially 16°C and increased to 17.5°C during the three hour trip to the field station. The fish were allowed to recover in 80 l tanks stocked at a density of 6-10 g fish per litre. Blood samples were taken before, and immediately after transportation, and after 12, 18, 36, and 84 hr of recovery.

STATISTICAL ANALYSIS

Results are presented as the mean ± S.E. Where appropriate, results were analyzed with Analysis of Variance (ANOVA) and the Duncan's Multiple Range Test. Some data were normalised with a logarithmic transformation before the statistical analysis was valid.

RESULTS

CONFINEMENT AND PHYSICAL DISTURBANCE

Both freshwater- and seawater-acclimated quinnat salmon showed a rapid and significant increase in plasma cortisol when confined and physically disturbed with a net ($P < 0.01$, Fig. 1A and 1B). The cortisol response in both cases peaked at 1 hr and then rapidly returned to control levels between 4 and 12 hr. However, changes in osmolarity, haematocrit, and sodium and chloride concentrations were different in the freshwater- and seawater-acclimated salmon. In fresh water, the confinement and agitation induced a significant decrease in plasma sodium and chloride concentrations, and blood osmolarity (Fig. 1A), whereas in seawater there was a significant increase in sodium and chloride levels and osmolarity. Both the absolute and percentage changes in sodium and chloride concentrations were greater in seawater-acclimated salmon. For both the seawater- and freshwater-

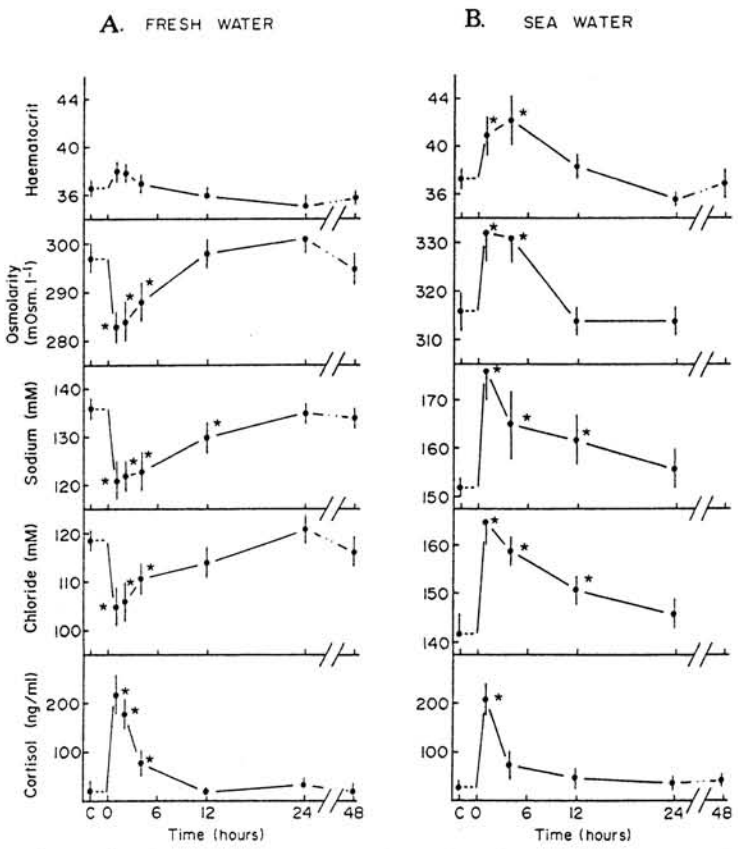

Figure 1. Changes in haematocrit and plasma variables of fish subjected to intense handling and confinement for 10 min. A. Freshwater acclimated quinnat salmon. B. Seawater acclimated quinnat salmon. Values are means ± S.E. (n = 4). Asterisks indicate mean values that are significantly different from initial values (Duncan's multiple range test, $P < 0.05$). C = control sample prior to handling and confinement.

acclimated salmon, the recovery time for the electrolytes was longer than for cortisol, with the electrolytes returning to baseline values between 12 and 24 hrs. Haematocrit increased significantly only in the seawater-acclimated salmon, increasing from 37.4 ± 0.6% to 42.2 ± 1.3%.

SEVERE HEAT STRESS

Freshwater-acclimated sockeye salmon exposed to a sudden heat shock displayed an increase in general activity and ventilation rate, and after 10 min at 35°C, several of the fish appeared exhausted and lay on the bottom of the aquarium with a slow, deep ventilatory rhythm. Physiologically, the salmon exhibited a marked increase in both haematocrit and plasma cortisol (Fig. 2). Plasma cortisol increased about 15-fold and peaked after 3 hours, returning to control levels in 24 hr. Haematocrit peaked after 1 hr and took 24 hr to return to baseline values.

SAMPLING FISH

There was no significant difference in the plasma cortisol titres of quinnat salmon sampled within 12 min of netting and anaesthesia (Fig. 3). However, 25 minutes after being netted and anaesthetized, the plasma cortisol concentration of quinnat salmon had increased to 48 ± 10 ng/ml cortisol, about a 3 - fold increase from the initial plasma cortisol levels. There was no change in plasma cortisol concentrations of sockeye salmon during the 25 min sampling period (Fig. 3). The elevation of plasma cortisol levels was faster and larger for quinnat salmon than for sockeye.

For both quinnat and sockeye salmon, there was no significant difference in the plasma cortisol concentrations of the fish sampled successively from the same aquarium at 0, 1, 4, 12, and 24 hrs (Fig. 4).

TRANSPORTATION

The quinnat and sockeye salmon did not appear unduly stressed after three hours of transportation. A few fish were taking the occasional

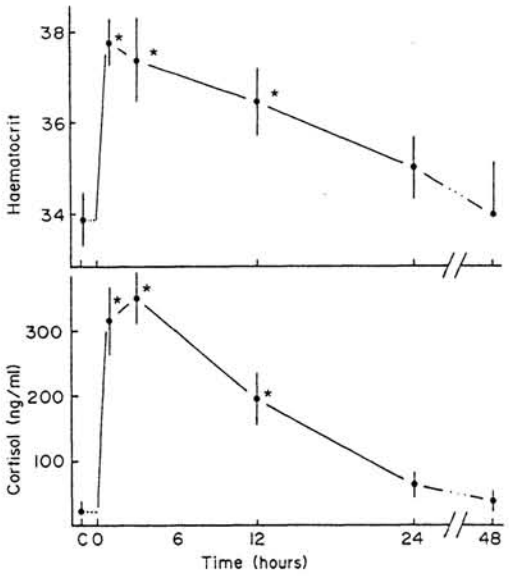

Figure 2. Changes in plasma cortisol concentrations and haematocrit of freshwater acclimated sockeye salmon subjected to a 10 min temperature stressor (an increase from 16°C to 35°C). Values are means ± S.E. (n = 3-6). C = control sample prior to temperature stressor. Asterisks indicate mean values that are significantly different from initial values (Duncan's multiple range test, $P < 0.05$).

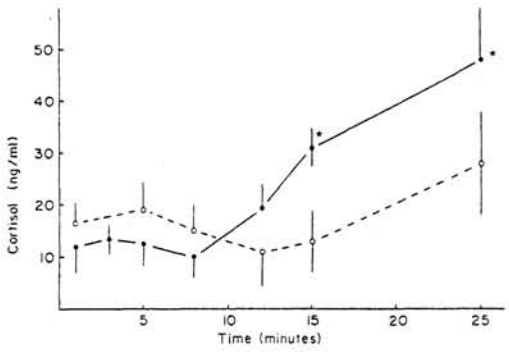

Figure 3. Changes in plasma cortisol levels of sockeye (o) and quinnat salmon (●) that have been netted and anaethetised with 2-phenoxyethanol. Values are means ± S.E. (n = 3). Asterisk indicates mean values are significantly different from initial value (Duncan's multiple range test, $P < 0.05$).

gulp of air at the surface, and some scale loss had occurred, but this was negligible. Transportation increased plasma cortisol concentrations by 100

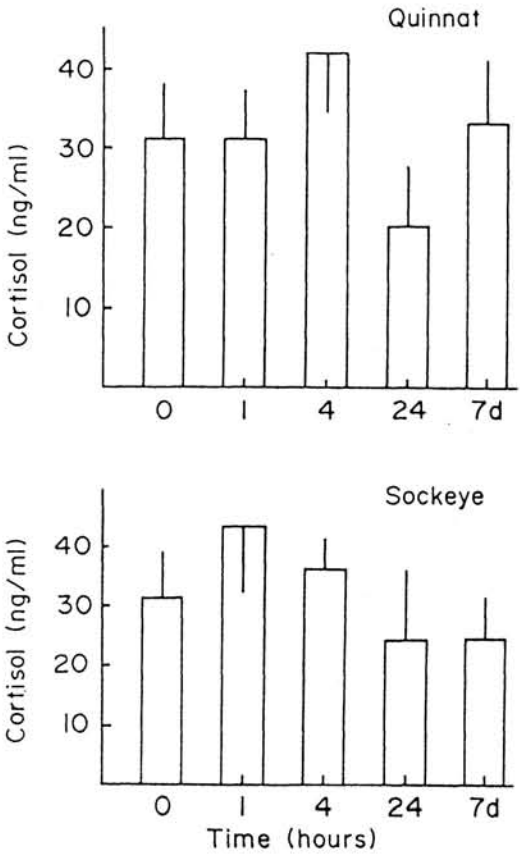

Figure 4. The effect of repeated sampling from an aquarium on the plasma cortisol levels of quinnat and sockeye salmon. Values are means ± S.E. (n = 5-7).

ng/ml in sockeye and 150 ng/ml in quinnat salmon. Plasma cortisol concentrations returned to control levels within 18 hr (Fig. 5). A greater elevation of plasma cortisol occurred in the quinnat salmon, and the quinnat generally appeared to be more distressed by transportation and handling. As in the freshwater confinement and agitation experiment, transportation of fish in fresh water caused a transient decrease in plasma chloride concentrations (Fig. 5). The recovery of plasma chloride took longer than blood cortisol, returning to baseline values within 36 hr.

DISCUSSION

A transient elevation of plasma cortisol resulted when sockeye and quinnat salmon were exposed to acute stressors. This primary re-

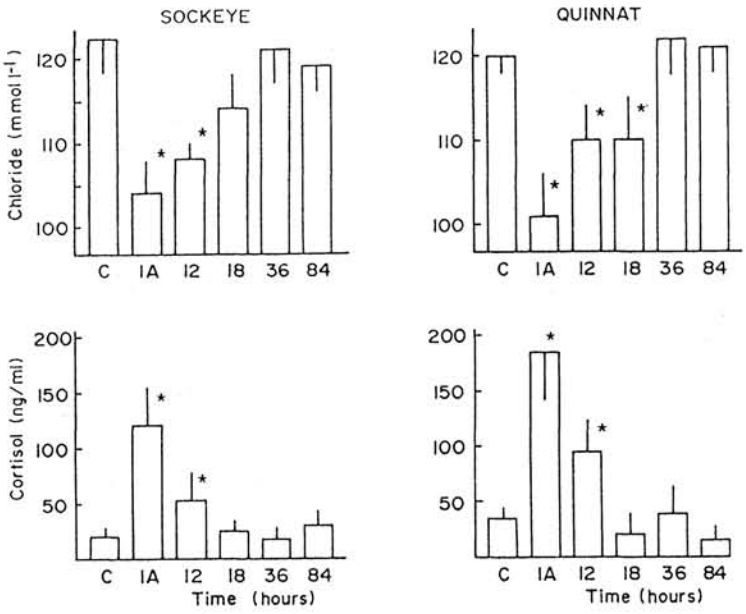

Figure 5. Changes in plasma cortisol and chloride concentrations in response to transportation followed by hours of recovery. Values are means ± S.E. (n = 4-7). Asterisks indicate mean values that are significantly different from initial values (Duncan's multiple range test, $P < 0.05$). C = control fish sampled immediately before the transportation. IA = fish sampled immediately after transportation.

sponse occurred regardless of the nature of the stressor, whether it was confinement, heat shock, transportation, or netting and anaesthesia. These findings support Selye's (1950) argument of a common physiological stress response, at least at the primary stress response level.

A rise in plasma cortisol has been reported to occur in a number of different teleost species exposed to a variety of different stressors (for review, see Pickering 1981). For this reason, cortisol has been widely used as an indicator of stress in fish. Donaldson (1981) went a step further and suggested that the response of the hypothalamic-pituitary-interrenal axis, which can be monitored by changes in plasma cortisol concentrations, could be used to quantify and compare different stressors. Differences in the magnitude and duration of the plasma cortisol elevation do occur, and this variation can be related in part to the type of stressor applied. The heat shock applied to sockeye salmon in the present study was considered to be the most severe stressor as it was the only treatment which produced mortality. This caused the greatest increase in plasma cortisol and had the longest recovery time. It is reported that generally, the more severe the stressor, the greater the cortisol elevation. For example, salmonids exposed to a range of concentrations of environmental pollutants or toxicants typically show a dose-dependant increase in the corticosteroid stress response (Donaldson & Dye 1975, Donaldson 1981). The magnitude of the physiological stress response is also affected by the number of stressors applied. Barton *et al.* (1986) showed that the physiological stress response of juvenile quinnat salmon is cumulative if the salmon are exposed to more than one stressor. The stress responses of teleosts have also been shown to be influenced by the water quality (Barton *et al.* 1985b), the health of the fish (Sumpter *et al.* 1986, Robertson *et al.* 1987) and the developmental stage (e.g., smoltification of salmonids, Barton *et al.* 1985a, Leatherland 1985, Specker & Schreck 1982).

Minor differences were noted between the stress response of quinnat and sockeye salmon in this study. The effect of transportation on sockeye salmon elicited a smaller primary stress response than for quinnat and their general behaviours were markedly different. During this study, the quinnat were more easily frightened or visibly seen to be stressed than sockeye salmon. This appears to be a characteristic of these two species in culture in New Zealand (P. Todd, NZMAF pers. comm.).

The haematocrit, plasma sodium and chloride concentrations and plasma osmotic pressure of quinnat salmon were altered by confinement

and agitation. These secondary stress responses had a smaller percentage change from pre-stressed values and generally had longer recovery times than the plasma cortisol changes associated with this stressor. An increase or decrease in plasma sodium and chloride concentrations osmotic pressure was dependent on the external salinity. In fresh water, salmon are hyperosmotic to the medium and hence ions presumably were lost down the concentration gradient while water gained by osmosis. Conversely, in sea water, salmon are hypoosmotic to the medium and so there was presumably an influx of ions and loss of water. A greater relative change in sodium and chloride concentrations occurred in the seawater acclimated salmon, possibly due to permeability differences of the gills in the two media. The osmotic and ionic changes could be the direct response to the stressor and/or the result of the adaptive mechanisms (i.e., the primary stress responses) initiated by the fish to combat the stressor. Mazeaud & Mazeaud (1981) suggest that the osmotic dysfunction that occurs when fish are stressed is due to the release of catecholamines which have a direct effect on the fish's branchial permeability to water and ions. Adrenaline, which is elevated during stress (Butler *et al.* 1978), has been shown to increase the permeability of the gill epithelium of teleosts to water and electrolytes (Pic *et al.* 1974, 1975). Cortisol may also affect the ionic and osmotic balance of fish, by behaving as a mineralocorticoid. It might be considered that the increase in plasma cortisol concentration following stress is caused by the osmotic dysfunction. However, Redding & Schreck (1983) found that an osmotic imbalance was not a necessity for a rise in plasma cortisol. They found that coho salmon placed into an isosmotic medium and then stressed by crowding showed no osmotic imbalance. Moreover, the time course of changes recorded in the present study suggests that cortisol peaks and returns to basal levels more rapidly than the ionic and osmotic concentrations.

Confinement and agitation caused an increase in the haematocrit of both the seawater- and freshwater-acclimated salmon, with a greater increase in the former group. The reason for the increase in haematocrit can not be determined without additional information. Soivio & Nikinmaa (1981) suggested that the elevation could be due to erythrocyte swelling and/or result from the stress related changes in the osmotic balance of the blood. The larger increase of haematocrit in the seawater acclimated quinnat could therefore be the result of both erythrocyte swelling and an efflux of water from the blood. In the freshwater acclimated quinnat, the smaller increase in haematocrit could have resulted from swelling of the erythrocytes being offset by a hemodilution of the blood through an osmotic influx of water. Beggs *et al.* (1980) found that in the freshwater muskellunge (Esox masquinongy), haematocrit levels initially rose, then subsequently fell after capture by angling. The reduced levels were the result of dilution of the blood.

Blood sampling-related stress responses were absent if the blood samples were taken within 10-12 min of netting and anaesthesia. Of the variables studied, plasma cortisol showed the shortest rise time and is therefore the variable that should be monitored during blood sampling. The influence of several types of anaesthetics and blood sampling methods on blood variables has been reported (Oikari & Soivio 1975, Strange & Schreck 1978, Wells *et al.* 1984). Strange & Schreck (1978) found that quinnat salmon anaesthetized rapidly with 100 mg/l MS-222 (ethyl m aminobenzoate methanesulfonate) showed no changes in plasma cortisol concentrations. The fish were exposed to MS-222 in their aquaria and were not netted out and placed into the anaesthetic as was done in this study. In the present study, cortisol levels were significantly elevated only after times greater than 12 min. As such, this elevation is likely to be a consequence of the long aerial exposure. As these represent extreme times for aerial exposure, under normal experimental circumstances it is not envisaged that fish would be left for such periods. Thus, for short time periods, anaesthesia and aerial exposure do not affect cortisol levels in salmon.

Transportation of quinnat and sockeye salmon affected plasma cortisol and chloride concentrations. Similar alterations in plasma cortisol and chloride levels as well as changes in haematocrit, plasma glucose, osmolarity and other electrolytes have been observed in fish af-

ter transportation (Carmichael *et al.* 1983, Robertson *et al.* 1987). The decrease in chloride concentration after transportation is part of a general osmotic imbalance of the blood, which includes changes in sodium concentration and osmolarity. This osmotic dysfunction has a significant effect on the subsequent survival of transported fish (Barton *et al.* 1980, Robertson *et al.* 1987, Specker & Schreck 1980). Transportation of fish in water that is isosmotic to fish blood has been found to negate disruption of the osmoregulatory homeostasis of the fish and significantly reduces mortality (Carmichael *et al.* 1984). However, in the present experiments there was no mortality during the transportation of the sockeye and quinnat salmon. This might be attributed to a low stocking density and short transportation time.

In summary, the rapid changes in the plasma cortisol concentrations of fish exposed to an acute stressor can be used effectively to characterise the initial effects of a stressor, but not the recovery from it. An indicator for recovery from a stressor, would be plasma chloride concentration.

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